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RESEARCH ARTICLE

Structure of the leaf litter frog community in an area of Atlantic Forest in southeastern Brazil

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ABSTRACT. Different spatial and temporal factors can influence the species richness and abundance of leaf anurans that are fundamental for the ecosystem functioning, as they act as predators and integrate the trophic chain as prey of other animals. There are relatively few studies that aimed to understand the spatio-temporal variation and the influence of environmental factors on leaf litter communities. We studied parameters of the anuran community living in the forest leaf litter in the Duas Bocas Biological Reserve (DBBR), Espírito Santo, Brazil. We sought to understand the extent to which richness, abundance, biomass and density varied between two locations with different stages of preservation (primary and secondary forest). In addition, we tested the effect of temperature and local humidity on abundance. We conducted the samplings monthly from October 2017 to September 2018, establishing 98 4 x 4 m plots (16 m^2 each) demarcated on the DBBR forest leaf litter. We measured temperature (°C) and relative air humidity (%), and each plot was carefully surveyed by four observers. We tested for differences in anuran density between the two sampled locations and estimated the effects of environmental variables in the community. We recorded 102 individuals of anurans from 11 species belonging to eight families. The DBBR anuran community parameters significantly differed between the two studied locations, with the highest values of anuran richness and abundance occurring in the area covered by primary forest, probably due to differences in the preservation of each area. However, temperature and humidity did not affect the abundance of anurans in the sampled areas. Our results provide the first information about spatial variation and influence of environmental factors, directed to the community of leaf litter anurans in DBBR, and represents the second study on this group of anurans in the state of Espírito Santo.

KEY WORDS. Amphibians, amphibian abundance, environmental variables, frog density, tropical forest.

INTRODUCTION

Amphibians of tropical forest leaf litter are fundamentally important for the functioning of the ecosystem because, as predators, they act to control the density of invertebrate populations, especially arthropods (Wyman 1998, Beard et al. 2002, Best and Welsh 2014) and, as preys, integrate the trophic chain with other animals such as birds, reptiles and mammals (Toft 1980, 1985, Toledo et al. 2007). These amphibians are indirectly linked to the dynamics of forest nutrients by controlling species that are responsible for starting the breakdown of organic material because, subsequently, microorganisms will consume and structurally modify this organic material, providing to the soil part of the nutrients necessary for primary productivity (Wyman, 1998, Beard et al. 2002, Huang et al. 2007).

Different spatial and temporal factors may influence species richness and abundance of anurans, such as temperature (Santos-Pereira et al. 2011), altitude (Scott 1976, Siqueira and Rocha 2013, Goyannes-Araújo et al. 2015), leaf litter depth (Scott 1976, Van Sluys et al. 2007 Oliveira et al. 2013, Siqueira and Rocha 2013), arthropod density available in forest leaf litter (Toft 1981), and relative air humidity (Oliveira et al. 2013). For example, altitude influences the variation of a set of biotic and abiotic factors, such as availability of food resources, air temperature, air humidity, the available area for occupation, number of water bodies, primary productivity, intensity of ultraviolet

radiation, among other factors (Siqueira and Rocha 2013). As a result, it is expected that the richness and composition of anuran species will change at different altitudinal levels (Vasconcelos et.al. 2010). In addition, a study carried out in different locations in Brazil found that the relative humidity and precipitation are the factors that best explained the variation between anuran communities (Vasconcelos et al. 2010).

Several studies carried out in different forest areas in the world provide data on the anuran amphibian communities that live in the leaf of the forest floor, such as on richness, composition, abundance, and density (e.g., Ryan et al. 2014, 2015, Whitfield and Pierce 2005, Watling and Donnelly 2002). In the Atlantic Forest, studies have shown between three and 16 anuran species in the leaf litter, in different altitudinal levels (e.g., Giaretta et al. 1999, Almeida-Gomes et al. 2010). However, there are relatively few studies with the aim of understanding the spatio-temporal variation and the influence of environmental factors on the leaf litter anuran communities in this biome. For example, Santos-Pereira et al. (2011) demonstrated that temperature significantly influenced the general abundance of leaf litter anurans in the Atlantic Forest of southern Brazil. However, in an area of Atlantic Forest in southeastern Brazil, only the humidity of the air and the depth of the litter have significantly influenced the local leaf litter anuran community (Oliveira et al. 2013).

Considering the importance and lack of information on anurans that occur in the litter of the Atlantic Forest, we aimed to study the parameters of the anuran community of leaf litter in the Duas Bocas Biological Reserve (DBBR) located in southeastern Brazil. We sought to specifically answer the following questions: 1) how is the DBBR leaf litter anuran community structured in relation to species richness, abundance, density, and biomass? 2) How does the anuran community vary spatially between the sampled areas of DBBR? 3) How does the anuran community vary between the dry and wet seasons in DBBR? 4) What are the effects of environmental variables (i.e. air temperature and air humidity) on the abundance of the leaf litter anurans? Our hypothesis is that the parameters of the leaf litter anuran community will differ between the sampled areas, with a higher richness in the primary forest area. We also expect differences in the species composition between the two areas. In addition, we expected the abundance of frogs to be higher in the rainy season, and that relative air humidity will more significantly influence the DBBR anuran community than air temperature.

MATERIAL AND METHODS

We conducted the study in the DBBR, located in the rural area of the municipality of Cariacica, with coordinates 20°14'04",20°18'30"S; 40°28'01", 40°32'07"W (Fig. 1), Espírito Santo, Southeastern Brazil.

The DBBR is a remnant of the Atlantic Forest, characterized as Submontane Dense Ombrophylous Forest, with an area of 2,910 ha. The elevation varies from 200 to 800 m above sea level, with high diversity of different groups of fauna and flora, holding rare and endangered species (Novelli 2010, Helder-José et al. 2016). The reserve is composed of a forest area with little evidence of disturbance or cutting (about 80% of the reserve; Boni et al. 2009, Helder-José et al. 2016) and a portion of secondary forest with about 50 years of regeneration, previously occupied by coffee (*Coffea arabica*, Rubiaceae) and mainly jackfruit plantations (*Artocarpus heterophyllus*, Moraceae) (Azevedo and Santos 2000). The DBBR climate is characterized as tropical humid with average annual temperature varying between 19.0–25.5 °C and average annual rainfall between 1500–1600 mm with relatively regular rainfall distribution throughout the year, with higher rainfall in summer (Bastos et al. 2015, Helder-José et al. 2016).

The DBBR has two main trails denominated the Represa Velha and Alto Alegre trails (Fig. 1). Represa Velha trail is 3500 m long, and is between 202 m and 213 m above sea level. It is composed of secondary forest area, where banana, coffee and pasture crops were introduced, in addition to a predominance of jackfruit, intercalated with other species in the forest (Boni et al. 2009, Novelli 2010). The site is surrounded by small and medium-sized rural properties (Boni et al. 2009) and is constantly visited for environmental education purposes.) During the study period, the mean air temperature of this region was 24.5 °C (21.4–28.2 °C) and the relative humidity of the air was on average 73.8% (52.8–82.0%). Alto Alegre trail is 4000 m long and is about 543 m above sea level. It is composed of forest with little evidence of disturbance, with a high density of large trees that provide a higher coverage of the leaf litter over the soil (Boni et al. 2009, Novelli 2010). During the study period, the mean air temperature of this region was 21.6 °C (18.3–24.6 °C) and the relative humidity of the air was on average 70.1% (43.3–100%).

We conducted field sampling monthly from October 2017 to September 2018, including the dry season (April to September) and rainy (October to March) months. We installed 98 leaf litter plots with 4 m^2 marked on the forest floor and surrounded by a one meter high plastic screen to prevent escape of anurans occurring inside the plot area (Allmon 1991, Rocha et al. 2000). We established 49 plots in each of the sampled areas (Represa Velha trail and Alto Alegre trail), randomly arranged, totaling 1568 m^2 of sampled area, maintaining a minimum distance of 50 m from the trail to minimize edge effect. To avoid possible effects of spatial pseudo-repetitions, we determined a minimum distance of 50 m between each of the plots and no point was sampled more than once. Of the 49 plots established in the Represa Velha trail, we sampled 19 plots in the dry season (304 m^2) and 30 in the rainy season (480 m^2). In the Alto alegre trail, we sampled 24 plots in the dry season (384 m^2) and 25 plots in the rainy season (400 m^2). The total sampled area considering the two areas was 688 m^2 in the dry season and 880 m^2 in the rainy season.

We installed the plots in the afternoon, before nightfall (2:00–3:00 pm). After nightfall (6:00–10 pm), we measured the

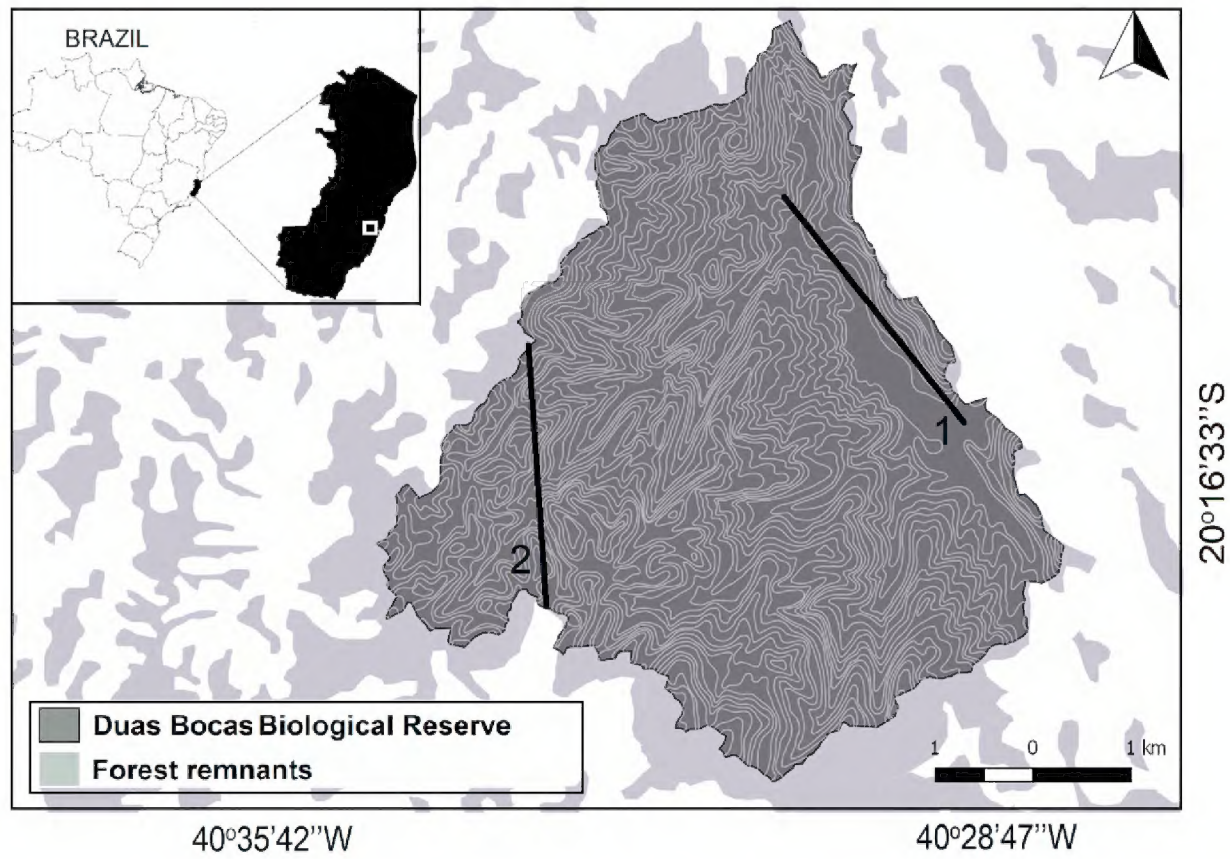


Figure 1. Location of the Duas Bocas Biological Reserve, Espírito Santo, southeastern Brazil. The black lines show the two localities sampled (1 = Represa Velha Trail, 2 = Alto Alegre Trail). Gray lines represent the contour lines of the area.

temperature (°C) and the relative air humidity (%) in each plot with a thermohygrometer. At the beginning of the sampling, the leaf litter in each plot was carefully surveyed by four observers using rattles and headlights, walking from one side to the opposite side of the plot in the same direction for 20 to 30 minutes depending on density of vegetation within the plots or the depth of the leaf litter. Each captured individual was placed in a plastic bag containing oxygen to avoid being sampled again and to allow the measurement of the body parameters. After the measurements, the individuals were returned to the interior of the plot where they were captured. We measured frog snout-vent length (SVL) measured in millimeters (mm), using a digital caliper and its mass measured with dynamometers Pesola® with capacity of 10 g (accuracy 0.1 g), 30 g (accuracy 0.5 g) and 100 g (1 g precision), depending on the size of the individual. We identified the found anurans to the lowest possible taxonomic level. We collected an individual of each species for species identification confirmation and deposited the vouchers as testimonial material (permit numbers IEMA-76433846 and SISBIO-56580-1) in the amphibian collection of the Museu Nacional do Rio de Janeiro (see supplementary file Table S1 for the voucher for anuran species collected). We euthanized specimens with anesthetic Lidocaine and fixed in 4% formalin solution. We estimated the density of each species in the community in each area by dividing the number of individuals by the sampled forest floor area, multiplied by 100 m^2 (frogs/100 m^2) and the total community biomass in each area based on the sum of the all individuals divided by 100 m^2. We used the same calculation to estimate the density of frogs in the dry and rainy seasons (i.e. the number of individuals divided by the area sampled in each season, multiplied by 100m^2), to minimize the effect of the effort difference in each season.

We checked our data for spatial autocorrelation with a Mantel test (Fortin and Dale 2005). In order to evaluate the extent to which the samples reached the predicted species richness of the area, based on the samples obtained as a function of the sampling effort (i.e. 98 plots) and the total sampling time, we calculated a species rarefaction curve using EstimateS 9.2 (Colwell 2009). We used the Chao 1 estimator, with 1000 randomizations, due to the relatively rare species quantity (Magurran 2013). For this analysis, we used the species abundance data, in each plot, throughout the study period.

We performed a Non-Metric Multidimensional Scaling (NMDS) of the Bray-Curtis index to compare the structure between the two areas in terms of species composition and abundance. The analyses were performed in R 3.4 (R Core Team 2018) using a "metaMDS" function in the Vegan package version 2.5-4 for community analysis (Oksanen et al. 2013). Bray-Curtis is a statistic used to quantify the compositional dissimilarity between two different sites, based on counts at each site, then it orders the objects based on the composition and abundance of the species (Kruskal 1964). Thus, the more similar two sites are in terms of species composition and abundance, the closer they will be in an ordination.

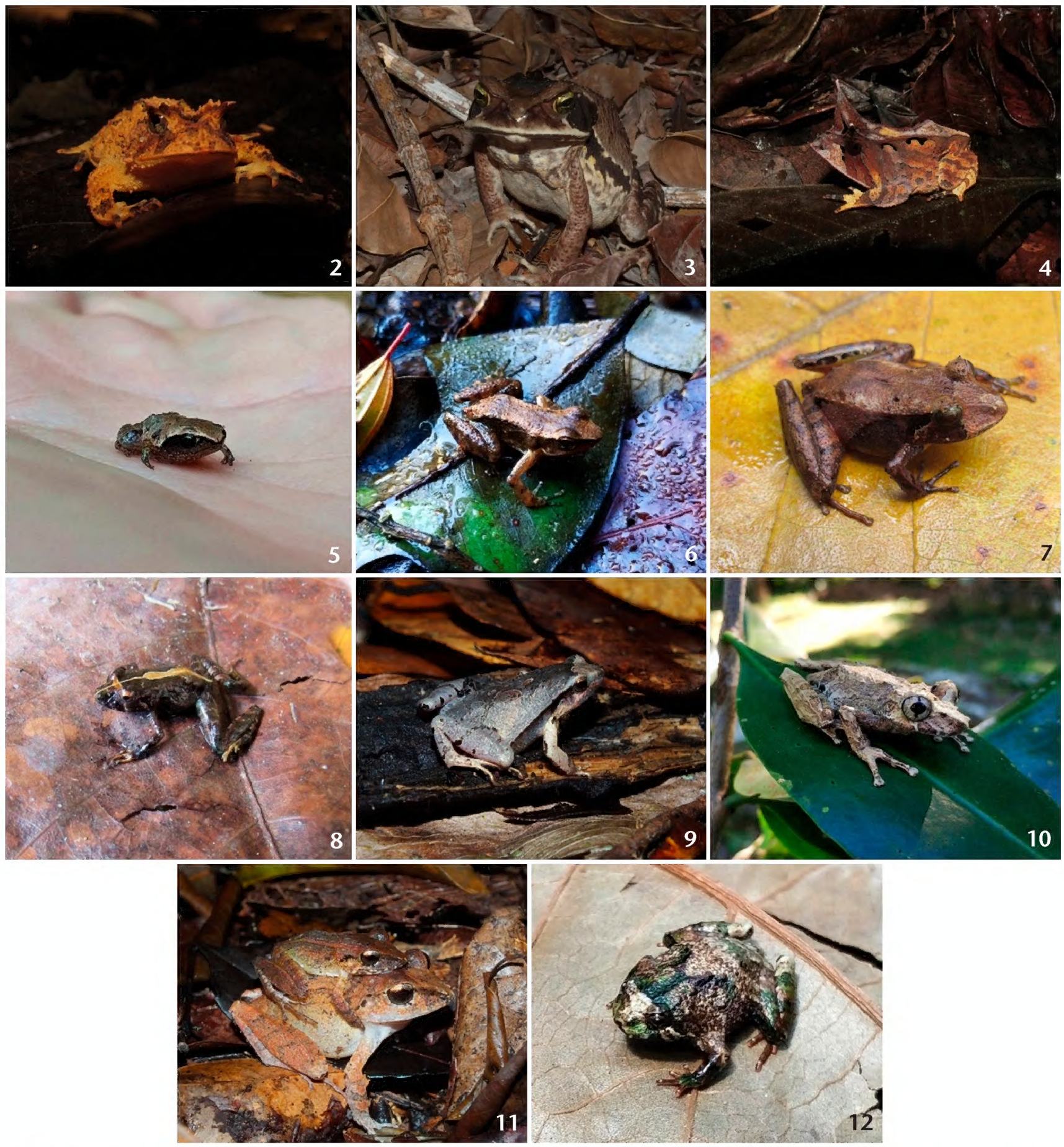
We tested the data for normality (Shapiro-Wilk normality test) and homogeneity of variance (Levene test). The differences were not statistically significant with $p > 0.05$. Thus, we performed a T test, based on species richness data for each plot in the two sampled areas to evaluate whether there were significant differences in the anuran community between the two areas.

Likewise, we performed a T test, based on the abundance data for each species in each season to evaluate whether there were significant differences in anuran density between the dry and rainy seasons.

We used Generalized Linear Models (GLMs) with a Poisson distribution and log-link function (Poisson regression) to analyze whether environmental variables (i.e. air temperature and humidity) influenced anuran density in each sampled area.

RESULTS

We captured 102 individuals from 11 species of anurans belonging to eight families, associated with the leaf litter (Table 1, Figs 2–12). We sampled 98 plots and 48.9% (48 plots) contained at least one individual, with a mean of 1.04 frogs/plot, 14 individuals being the maximum number found in one plot. The most representative families were Craugastoridae,



Figures 2–12. Species of anurans found in the Duas Bocas Biological Reserve, Cariacica, Espírito Santo: (2) *Proceratophrys schirchi*; (3) *Rhinella crucifer*; (4) *Proceratophrys laticeps*; (5) *Euparkerella tridactyla*; (6) *Crossodactylus* aff. *gaudichaudii*; (7) *Ischnocnema oea*; (8) *Ischnocnema abdita*; (9) *Physalaemus crombiei*; (10) *Ololygon kautskyi*; (11) *Haddadus binotatus*; (12) *Zachaenus carvalhoi* .

Table 1. Species of anurans recorded in the forest leaf litter of the Duas Bocas Biological Reserve, Cariacica, Espírito Santo, Brazil. (A) Abundance, (D) density (frogs/100 m^2), (B) biomass (g/100 m^2), (NT) Near Threatened (IUCN), (VU) Vulnerable (IUCN), (DD) Data deficient (IUCN).

Species	Represa Velha Trail			Alto Alegre Trail			Total		
	A	D	B	A	D	B	A	D	B
Bufonidae									
Rhinella crucifer (Wied-Neuwied, 1821)	41	5.23	2.845	5	0.64	0.482	46	2.93	3.327
Brachycephalidae									
Ischnocnema oea (Heyer, 1984)^{NT}	–	–	–	4	0.51	0.052	4	0.26	0.052
Ischnocnema abdita (Canedo & Pimenta, 2010)	1	0.13	0.006	–	–	–	1	0.06	0.006
Craugastoridae									
Haddadus binotatus (Spix, 1824)	4	0.51	0.266	20	2.55	0.812	24	1.53	1.078
Euparkerella tridactyla (Izecksohn, 1988)^{VU}	–	–	–	3	0.38	0.014	3	0.19	0.014
Cycloramphidae									
Zachaenus carvalhoi (Izecksohn, 1983)^{DD}	–	–	–	1	0.13	0.01	1	0.06	0.01
Hylidae									
Ololygon kautskyi (Carvalho e Silva & Peixoto, 1991)^{DD}	2	0.26	0.04	–	–	–	2	0.13	0.04
Hylodidae									
Crossodactylus aff. *gaudichaudii* (Duméril & Bibron, 1841)	–	–	–	2	0.26	0.061	2	0.13	0.061
Leptodactylidae									
Physalaemus crombiei (Heyer & Wolf, 1989)	2	0.26	0.018	3	0.38	0.033	5	0.32	0.051
Odontophrynidae									
Proceratophrys schirchi (Frank & Ramus, 1995)	–	–	–	13	1.66	0.253	13	0.83	0.253
Proceratophrys laticeps (Izecksohn & Peixoto, 1981)	–	–	–	1	0.13	0.039	1	0.06	0.039
Total	50	6.38	3.18	52	6.64	1.76	102	6.50	4.93

Brachycephalidae and Odontophrynidae, with two species each. The rarefaction curve, generated for all sampled plots, tended to stabilize, indicating a predicted species richness of about 13 species for the DBBR area (Bootstrap = 12.97 ± 1.6).

In general, the species with highest abundance and density in DBBR were *Rhinella crucifer* (Wied-Neuwied, 1821) (45%), followed by *Haddadus binotatus* (Spix, 1824) (23.5%). The species with the lowest abundance and density was *Ischnocnema abdita* (Canedo & Pimenta, 2010) (0.98%) (Table 1). The estimated total density for this community was 6.50 frogs/100 m^2.

The highest biomass was for *R. crucifer* (67.5%), followed by *H. binotatus* (21.7%) and the lowest was for *I. abdita* (0.12%). The estimated total biomass for this community was 4.93g/100m^2 (Table 1).

There was no significant spatial autocorrelation (R^2 = 0.072, p = 0.09) between occurrences of anurans and the spatial locations of sampling plots. The anuran community varied between the two areas (Fig. 13) and the difference in anuran richness significantly varied between areas (T-test, T = 2.062, DF = 96, p = 0.04).

We recorded five species in the Represa Velha trail, being *R. crucifer* the species with higher abundance in this area. In the Alto Alegre region, we recorded nine species, being *H. binotatus* the most abundant in this area (Fig. 14).

The anuran community varied between the two seasons (Table 2), but the density of anurans did not differ significantly between the dry and rainy seasons (T test, T = 0.443, DF = 18, p = 0.66) and the environmental variables did not significantly affect the density of frogs in the two sampled areas (Table 3).

Table 2. Comparison between abundance and estimated density (frogs/100 m^2) of leaf litter anurans found in the two sampled areas, in the dry and rainy seasons, in the Duas Bocas Biological Reserve, Brazil.

Species	Represa Velha Trail		Alto Alegre Trail		Total	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Rhinella crucifer	10	31	3	2	13	33
Ischnocnema oea	0	0	2	2	2	2
Ischnocnema abdita	1	0	0	0	1	0
Haddadus binotatus	3	1	10	10	13	11
Euparkerella tridactyla	0	0	2	1	2	1
Zachaenus carvalhoi	0	0	0	1	0	1
Ololygon kautskyi	2	0	0	0	2	0
Crossodactylus aff. *gaudichaudii*	0	0	2	0	2	0
Physalaemus crombiei	2	0	0	3	2	3
Proceratophrys schirchi	0	0	6	7	6	7
Proceratophrys laticeps	0	0	0	1	0	1
Total	18	32	25	27	43	59
Density (frogs/100m^2)	5.92	6.67	6.51	6.75	6.25	6.7

Table 3. Coefficients from Generalized Linear Models (GLM) with the effect of air temperature and relative humidity on the density of leaf litter anurans in the Duas Bocas Biological Reserve, Espírito Santo, Brazil.

	Estimate	Std. error	Z value	p
Represa Velha trail (intercept)	1.98267	2.99640	0.662	0.508
Air temperature	-0.03070	0.09012	-0.341	0.733
Humidity	-0.01757	0.03101	-0.567	0.571
Alto Alegre trail (intercept)	0.372104	1.972941	0.189	0.850
Air temperature	-0.015058	0.103315	-0.146	0.884
Humidity	-0.000672	0.013558	-0.050	0.960

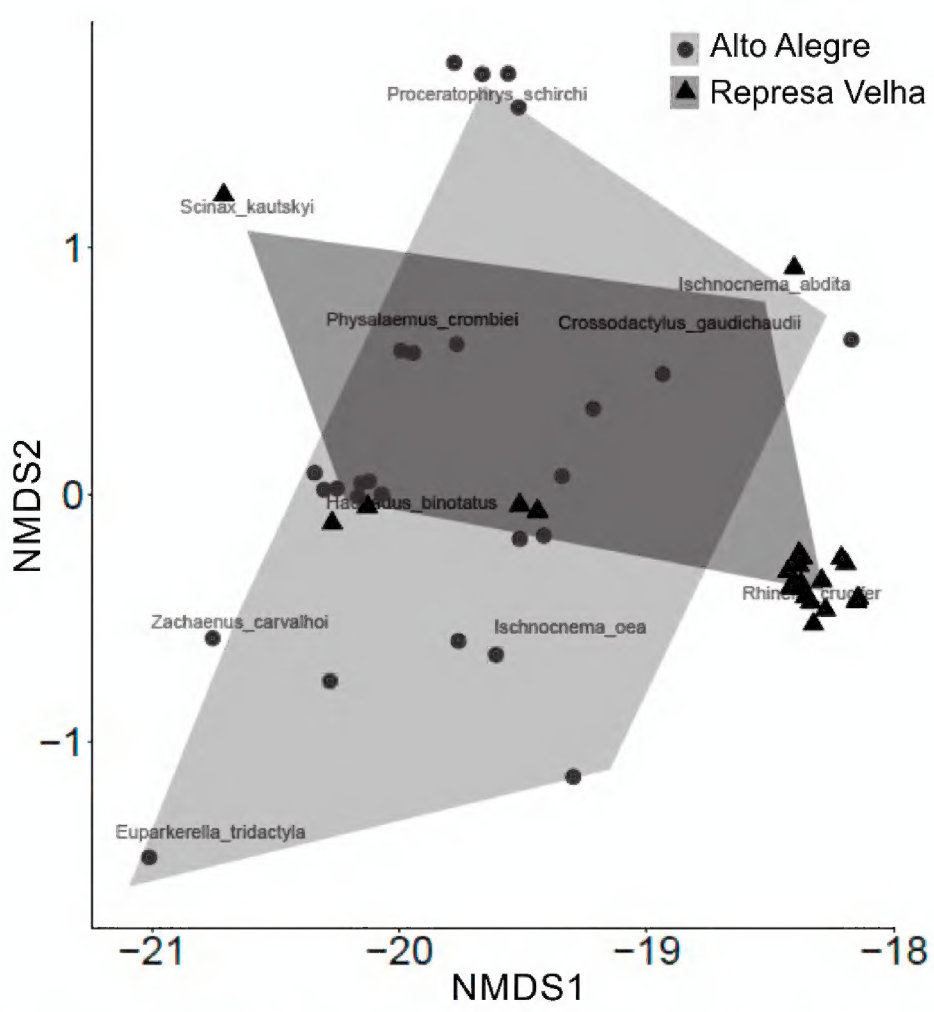


Figure 13. Non-Metric Multidimensional Scaling (NMDS) showing the similarity of the leaf litter anuran community in two areas of the Duas Bocas Biological Reserve. The points represent the plots sampled in the two areas between October 2017 to September 2018, with the circles representing the plots arranged in the Alto Alegre area and the triangles representing the plots arranged in the Represa Velha area.

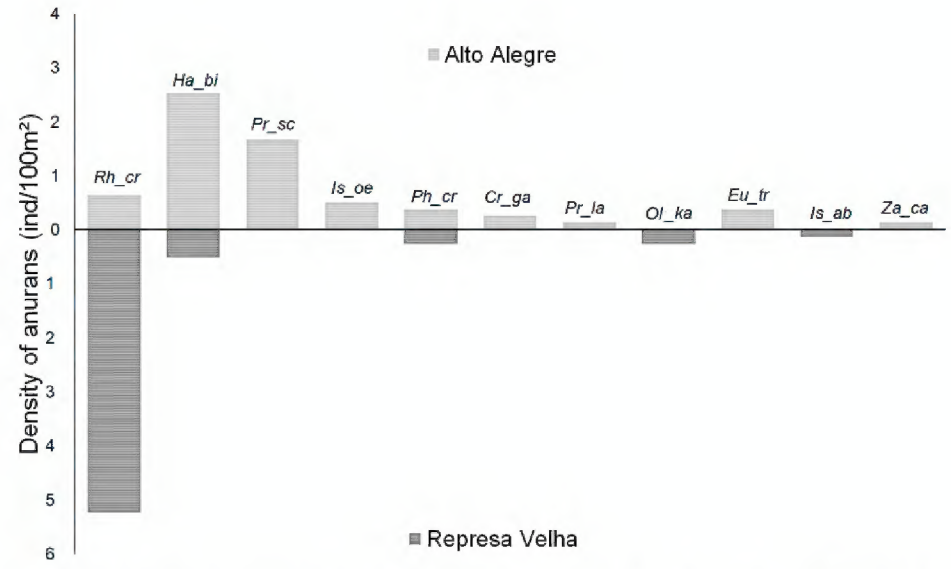


Figure 14. Density of forest leaf litter anurans by locality in the Duas Bocas Biological Reserve, Espírito Santo, Brazil. (Rh_cr) *Rhinella crucifer*, (Ha_bi) *Haddadus binotatus*, (Pr_sc) *Proceratophrys schirchi*, (Is_oe) *Ischnocnema oea*,(Ph_cr) *Physalaemus crombiei*, (Cr_ga) *Crossodactylus gaudichaudii*, (Pr_la) *Proceratophrys laticeps*, (Ol_ka) *Ololygon kautskyi*,(Eu_tr) *Euparkerella tridactyla*, (Is_ab) *I. abdita*, (Za_ca) *Zachaenus carvalhoi.*

DISCUSSION

We found that the species richness of the DBBR leaf litter anuran community is composed by 11 species. In general, the species richness found in the present study was higher than the results found for leaf litter anurans in other areas of the Atlantic Forest in Brazil (Table 4) (e.g., Rocha et al. 2001, 2007, 2010, Siqueira et al. 2009, Santos-Pereira et al. 2011). The rarefaction curve showed a tendency to stabilize, and predicted a richness of 13 species for our study area in the DBBR. This shows that our sampling effort was satisfactory and we sampled a species richness close to that predicted for the area (85% of the richness expected to occur in the area, or 77% of the richness if we do not consider *Ololygon kautsky* (Carvalho e Silva & Peixoto, 1991), a species not typical of leaf litter). Basically,our study did not find only two expected species for the area, and this may be related to the absence of records of species that have their ecology associated with specific conditions, such as species that have explosive breeding in a short period of time after heavy rains, such as the species of Microhylidae (Wells 1977). In fact, there are records of species of this family in DBBR (Tonini et al. 2010) that were not found in this study. Two individuals of the arboreal species (*O. kaustsky*) we found were in a plot close to a creek. This occurrence may have resulted from the fact that leaf litter is used when close to streams or that during sampling individuals moved from low shrubs within the plots to the leaf litter when disturbed by the samplers during sampling.

The data showed that the most representative families in terms of species richness were Craugastoridae, Brachycephalidae and Odontophrynidae. This result is similar to those of other studies in other areas of Atlantic Forest, and other Neotropical leaf litter communities, where the most representative family was Brachycephalidae (e.g., Rocha et al. 2007, Almeida-Gomes et al. 2008, Siqueira et al. 2009, Santos-Pereira et al. 2011, Oliveira et al. 2013).

The general biomass of leaf litter anurans recorded in DBBR showed higher values (Table 1) compared to most studies on leaf anurans in the Atlantic Forest area except for a few studies (e.g., Siqueira et al. 2009, Santos-Pereira et al. 2011). According to Siqueira et al. (2009) differences in biomass between different areas are related to the composition of communities of each locality. In fact, in the studied area of the DBBR, we recorded the occurrence

Table 4. Comparison between richness and estimated density (individuals/100 m^2) of anurans found in the Atlantic Forest leaf litter by sampling methodology in plots.

Location	Altitude (m)	Richness	Density (ind/100 m^2)	Author
Reserva Biológica Duas Bocas/ES	173–655	11	6.5	Present Study
Serra das Torres/ES	500–900	14	6.6	Oliveira et al. 2013
Reserva Nat. Salto Morato/PR	200–300	7	3.73	Santos-Pereira et al. 2011
Monte Verde Cambuci/RJ	100–650	3	3.1	Almeida-Gomes et al. 2010
Estação Ecol. Est. Paraíso/RJ	20–1350	8	4.3	Rocha et al. 2010
Parque Est. Três Picos/RJ	500–800	7	17.1	Siqueira et al. 2009
Morro São João/RJ	10–320	6	4.5	Almeida-Gomes et al. 2008
Reserva Ecol. Guapiaçu/RJ	40–400	8	4.8	Rocha et al. 2007
Ilha Grande/RJ	220–230	9	5.9	Rocha et al. 2001
Parque Flor. De Itapetininga/SP	900–1250	16	4.6	Giaretta et al. 1999
Serra do Japi/SP	850–1000	4	2.7	Giaretta et al. 1997

of larger species, unlike Rocha et al. (2007) where the abundance of small species was higher than those of considerably larger size.

Rhinella crucifer was responsible for about 68% of the total biomass of the anuran community of DBBR litter, which is directly related to its larger size in relation to the other species found. In addition, our results showed that *R. crucifer* was the species with the highest density in the leaf litter community of DBBR. In fact, it has been shown that species of the Bufonidae family are commonly more abundant in studies carried out with leaf litter amphibians (Dixo and Verdade 2006, Pontes and Rocha 2011). The high abundance of anuran species that live in leaf litter may be related to the reproductive period of the species, when sampling coincides with the period when there should be a high concentration of individuals in certain locations (Pontes and Rocha 2011). Probably the high density of *R. crucifer* in the present study is related to this reproductive period, since we registered a high number of juveniles' individuals in a few plots. For example, the largest number of individuals recorded on a plot was 14 individuals, all of whom were juveniles from *R. crucifer*. In addition, *R. crucifer* was the species that most varied in relation to abundance between the dry and rainy seasons in the two sampled areas.

Another species that had high density and biomass in DBBR was *H. binotatus*, similar to other studies in the Atlantic Forest (e.g., Almeida-Gomes et al. 2008, 2010, Rocha et al. 2010). In contrast to *R. crucifer*, this species has direct development and reproduction that is independent of water bodies (Haddad et al. 2013). In this sense, Pontes and Rocha (2011) indicate that the dominance of species with direct development (e.g., *Brachycephalus* spp., *H. binotatus*, *Ischnocnema guentheri*), may be directly related to the independence that these species have in relation to water bodies. The general density of *H. binotatus* (1.53 ind/100 m^2) had higher values compared to some other leaf litter anuran studies in Atlantic Forest areas (e.g., 0.23 ind/100 m^2, Santos-Pereira et al. 2011) but lower than other studies (e.g., 3.4 ind/100 m^2, Siqueira et al. 2014; 4.5 ind/100 m^2, Almeida-Gomes et al. 2008). However, when verifying density values, we must consider the variation that occurs due to the size of the plots used and the sampling period, even in the same region (Pontes and Rocha 2011).

We found differences in composition, species richness and density of species of anurans between the two studied localities (Figs 13, 14). The variation in these community parameters should be related to the different degrees of conservation of the forest in each area and with the differences in environmental conditions between the study sites (e.g., altitude), which may have influenced the richness. For example, the Represa Velha area, which has a lower species richness, is an area that has secondary forest and exotic species of flora, such as jackfruit (Boni et al. 2009). These characteristics may favor the occurrence of generalist species. In fact, in the area occupied by jackfruits, species richness was lower, with only the occurrence of *R. crucifer* in higher abundance, which is considered a common species in disturbed environments (Aquino et al. 2004). We have also sporadically recorded some specimens of *H. binotatus*, because this species also occurs in secondary forests (Condez et al. 2009).

On the other hand, the Alto Alegre area had higher species richness, which may be related to the fact that this area presents little or no evidence of disturbance, with a high density of trees providing higher coverage of leaf litter on the ground. In addition, the Alto Alegre area is located at altitudes above 540 m above sea level and, in general, altitude is known to be a favorable condition for some species of leaf litter anurans (Rossa-Feres et al. 2018). In fact, in our study, two species were found only in the Alto Alegre area, *Euparkerella tridactyla* (Izecksohn, 1988), which occurred at altitudes of 648 m above sea level, also recorded at high altitudes in other regions (e.g., Izecksohn 1988, Ferreira et al. 2010), and *Ischnocnema oea* (Heyer, 1984) that occurred between 560–655 m above sea level, as recorded in other works (e.g., Condez et al. 2009, Tonini et al. 2010, Montesinos et al. 2012).

Of the species recorded in the present study, two are classified as threatened by the International Red List of Threatened Species (IUCN), being classified as Vulnerable (*E. tridactyla*) and Near Threatened (*I. oea*) and two are classified with insufficient data to assess the conservation status: *Ololygon kautskyi* (Carval-

ho e Silva & Peixoto, 1991) and *Zachaenus carvalhoi* (Izecksohn, 1983). Of these four species, three were recorded only in the Alto Alegre area, highlighting the importance of conservation of the area as a whole.

The temperature and humidity did not influence the abundance of the anurans, an opposite result to our initial hypothesis (Table 3). Furthermore, the anuran community did not significantly differ between the dry and rainy seasons. This may be because the DBBR receives considerable levels of rain during all months of the year, and the temperature and humidity remained stable and did not significantly affect the abundance of the leaf litter anuran community. Bastos et al. (2015) demonstrated that the Duas Bocas basin receives rainfall throughout the year and does not have months without rainfall, but in some of them, rainfall occurs with higher values. This higher incidence of rainfall starts in October with the values increasing gradually until December where it reaches the highest values in general, approaching a rainfall pattern over the years for the area (Bastos et al. 2015). The area where the DBBR is located is associated to the morphology of the Patamares Escalonados Sul Capixaba, being the highest area of the Duas Bocas Hydrographic Basin, and these higher rainfall indices indicate the presence of the orographic effect that cooperates to increase the total rainfall in the region (Bastos et al. 2015).

We conclude that the parameters of the leaf litter anuran community of DBBR differed in relation to the two studied localities, probably due to differences in the state of preservation of the areas, with higher values of richness and abundance in the Alto Alegre area, which is covered by primary forest and without presence of exotic species of flora. In addition, two of the species that are currently categorized as threatened by IUCN were recorded only in the Alto Alegre area, emphasizing the importance of preserved areas for species conservation. Our study showed that temperature and humidity did not affect the abundance of anurans in the sampled area and it is probably related to the high rainfall indexes of the region throughout the year, which is favorable for most species of anurans. Our study provides information on the spatial and temporal variation and influence of environmental factors on the leaf litter anuran community in the DBBR and our results provide important information that can be used in the planning of conservation actions of the Atlantic forest.

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Supplementary material 1

Table S1. Voucher for anuran species collected at the Duas Bocas Biological Reserve, Espírito Santo, and deposited in the amphibian collection of the Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil.

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Data type: species data.

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